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A Single Nucleotide Polymorphism in Transforming Growth Factor-β type II Receptor of the Rat

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ABSTRACT *: A single nucleotide polymorphism in the transforming growth factor-β type II receptor (TGFβRII) gene of the rat was studied. TGFβRII is a tumor suppressor that is frequently inactivated by mutation in human colon cancers. A novel nucleotide polymorphism of G to A (or A to G), which causes a silent mutation at codon 129, was found in G:C rich sequence in the TGFβRII gene of Sprague-Dawley rats. The results suggest that genetic polymorphism occurs within a strain of the laboratory animal.*

Key Words *: Transforming growth factor-β type II receptor, Nucleotide polymorphism*

I. INTRODUCTION

Transforming growth factor-β (TGFβ) regulates a variety of cellular processes including cell proliferation, extracellular matrix formation, apoptosis, migration and adhesion (Sporn *et al.*, 1987: Lyons *et al.*). TGFβ is also involved in embryonic morphogenesis. tissue differentiation, and wound healing. The physiology of TGFβ is influenced by various factors, including levels of TGFβ and presence of other growth factors. At least nine different cellular TGFβ binding proteins have been identified, showing different patterns of expression in different tissues. TGFβ signal transduction is primarily mediated by a heteromeric complex of two related serine-threonine kinases, Type I (TGFβRI) and Type II (TGFβRII). A cascade of interactions is involved in TGFβ signaling: ligand binding to TGFβRII, formation of a heterodimer complex between TGFβRI and TGFβRII, transphosphorylation of TGFβRI by TGFβRII, and initiating downstream signaling (Massague, 1998). Thus, the TGFβRII appears to be essential for the biological activity of TGFβ. TGFβ receptor type III (TGFβRIII), although capable of binding to TGFβR, is distinct from TGFβRI and TGFβRII and has no kinase domain. TGFβRIII has been thought to associate physically with TGFβRI-TGFβRII signaling complex and facilitate ligand binding to TGFβRII (Lopez-Casillas *et al.*, 1993).

Microsatellite instability (MSI) involves misalignment mutagenesis and alterations in the lengths of simple repetitive sequences (Loeb, 1998; Duval *et al.*, 1999). MSI, well studied in hereditary nonpolyposis colorectal cancer, is associated with defects in DNA mismatch repair. One of the examples of MSI is *TGFβRII* frameshift mutations clustered within a microsatellite-like 10 base pair polyadenine repeat within the coding region (BAT-RII tract). Such BAT-RII tract frameshift mutations are frequently found in colon cancers that arise by the pathway of MSI, and biallelic inactivation of *TGFβRII* secondary to BAT-RII mutations can be found in 90% of MSI colon cancer (Parsons *et al.*, 1995).

Genetic polymorphism in *TGFβRII* in the rat was examined. The current study demonstrates that *TGFβRII* of Sprague-Dawley rats has a nucleotide polymorphism in G : C rich sequence.

II. MATERIALS AND METHODS

Genomic DNA isolation: Genomic DNA of mammary glands and kidneys from 25 week-old Sprague-

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ABBREVIATIONS
TGFβRII, transforming growth factor-β type II receptor; TGFβ, transforming growth factor-β; MSI, Microsatellite instability; PCR-SSCP, Polymerase chain reactions-single strand conformation polymorphism

Dawley and Fisher344 rats were isolated by phenol/chloroform extraction. Briefly, tissues were homogenized in 10 mM Tris-HCl and 1 mM EDTA (TE), pH 7.6. The homogenate was incubated with RNAse for 3 hour at 37°C, followed by an overnight incubation with proteinase K (100 mg/ml) in the presence of 1% sodium dodecyl sulfate. The lysate was then extracted with phenol/chloroform. The DNA was precipitated with ethanol, washed with 70% ethanol and resuspended in TE buffer.

Polymerase chain reactions-single strand conformation polymorphism (PCR-SSCP): A set of allele specific PCR primers was designed with the GCG Program (Genetics Computer Group, Madison, WI). The primer sequences are as follows: sense CCCAAGTTCAGGTACCA and antisense CAGGAGCACATGAAGAA. PCR amplication was performed with Taq polymerase in the presence of [^{33}P]αATP (0.1 μCi/μl). After PCR, 1 μl of the reaction mixture was diluted with 9 μl of loading buffer (95% formamide, 20 mM EDTA) and heated at 94°C for five minutes prior to being incubated on ice. Subsequently 1 μl was loaded onto a 6% polyacrylamide gel containing 0.5× TBE and 5% glycerol. Electrophoresis was performed for four hours at 4°C. The gel was then dried and visualized by autoradiography.

Direct nucleotide sequencing: Bands on the SSCP gel were cut out, eluted with 50 μl of water, and used as the templates of PCR with nonlabeled primers. After PCR, the reaction mixture was gel-purified with Qiaex II Gel Extraction Kit (Qiagen). The PCR product was then labeled using ABI Prism Dye Terminator Sequencing Ready Reaction Kit Nucleotide sequencing was performed with ABI373 autosequencer (Perkin-Elmer).

II. RESULTS AND DISCUSSION

Allele specific PCR-SSCP analysis for *TGFβRII* in Sprague-Dawley rats showed three types of band patterns: H1, H2 and H3 (Fig. 1). In contrast, only H1-type was found in the DNA samples from Fisher344 rats. Based upon direct sequencing (Fig. 2), the bands in H1-type lanes represent an allele that was previously reported (GenBank Accession S67770). The bands in H2-type lanes contain a single nucleotide substitution of G : C to A : T in G : C rich sequence.

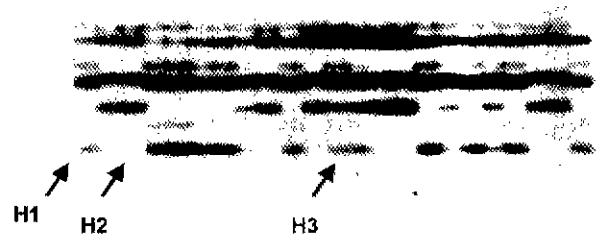

Fig. 1. PCR-SSCP analysis of a nucleotide substitution in the *TGFβRII* gene in genomic DNA samples of Sprague-Dawley rats. H1, H2 and H3 represent three different types of band patterns in the polyacrylamide gel.

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      LysGluLysLysArgAlaGlyGluThr
621 aaggaaaagaaaagggcggcgagacc 647
                    |
      aaggaaaagaaaaggGcAggcgagacc
      LysGluLysLysArgAlaGlyGluThr
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Fig. 2. A single nucleotide polymorphism of *TGFβRII* of the rat. The sequence of upper DNA strand is from GenBank Accession S67770. A novel nucleotide polymorphism of G to A (or A to G) at nucleotide 387 is indicated in upper case.

The nucleotide substitution, however, is a silent mutation (Fig. 2). H3-type lanes represent a heterozygote of H1 and H2. Among the 23 samples used for PCR-SSCP analysis, H1-, H2- and H3-types occur at the frequencies of 10/23, 8/23 and 5/23, respectively (Fig. 1).

Microsatellites nearly always occur at noncoding sequences with no known function, and mutations of these may be regarded as neutral. In this case, the mutant microsatellite alleles, which accumulate by chance as a result of an increased mutation rate can be regarded as bystander mutations. Short runs of mono- or dinucleotide repeat sequence can also be found in the coding regions of certain tumor suppressor genes such as *BAX*, *E2F4*, insulin-like growth factor II receptor and in the human. Since frameshift mutations of the genes results in altered gene products, they have been assumed to give an selective advantage (Rampino *et al.*, 1997).

In humans, the *TGFβRII* gene contains a mononucleotide $(A)_{10}$ repeat within the 5′ end of the coding region that appears to be a consistent target for inactivating insertion/deletion mutations in gastrointestinal cancers deficient in mismatch repair. A $(GT)_3$ repeat within 3′ end of the coding region of *TGFβRII* is also a target of mutational inactivation in mismatch

repair deficient human colon cancers (Yamamoto *et al.*, 1997).

In contrast to a mononucleotide $(A)_{10}$ repeat of human *TGFβRII*, rat *TGFβRII* contains an incomplete repeat, -AAAAGAAAA-. In the present study, a base substitution of G : C to A : T was found close to the incomplete repeat (Fig. 2). The mechanism by which the two allele coexists in the outbred strain is unclear. The base substitution causes a silent mutation and does not affect physiological functions of the gene product. However, it should be noted that there exists genetic variability within a strain of the laboratory animal used for research purpose.

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